

## MAINTENANCE AND MATURATION OF SQUID (*ILLEX ILLECEBROSUS*) IN A 15 METER CIRCULAR POOL

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Most interest in the laboratory maintenance of squid has centered on their use as a source of giant nerve fibers for neurophysiological studies. Although a number of smaller, partially benthic, cephalopods have been successfully reared (see Boletzky, 1974, and Gabe, 1976, for reviews), there is, as yet, no standard laboratory cephalopod; and long term maintenance of the larger teuthids with giant axons remains a problem. Summers, McMahon and Ruppert (1974), having evaluated a number of factors contributing to the mortality of *Loligo pealei* in captivity, conclude that skin damage resulting from contact with tank walls is a major factor in their limited survival (15 days maximum mean survival time) in small (1.68 m<sup>2</sup> area) rectangular tanks. Mikulich and Kozak (1971) and Matsumoto (1976) have reported slightly longer survival of *Todarodes pacificus* and *Doryteuthis bleekeri*, respectively, in larger (25 m<sup>2</sup> area) and circular (1.76 m<sup>2</sup> area) tanks. For *Illex illecebrosus* in captivity at the Marine Sciences Research Laboratory, Newfoundland, typical survival times of one to two weeks have been reported (Rowe and Mangold, 1975), but recently with larger tanks some animals have survived for 60 to 80 days (C. C. Lu, Memorial University of Newfoundland, personal communication). While *I. illecebrosus* is not used for giant axon studies, it is similar in size and behavior to the species that are, and the results of this study suggest that, given a healthy starting stock and an adequate volume of sea water, long term maintenance of this species and probably others is possible.

The present experiments were undertaken to determine whether chronic studies on the endocrinology of *I. illecebrosus* were feasible using the 15 m diameter tank in the Aquatron Laboratory at Dalhousie University and to learn more about the species' reproductive biology. Information on the reproduction and life cycle of *I. illecebrosus* should be of value in rationalizing an increasing commercial fishery (Mercer, 1973a). This report deals primarily with survival and with techniques suited to chronic studies; however, maturation is also discussed since most cephalopods die shortly after spawning, and degenerative changes associated with maturation are likely to influence survival.

### MATERIALS AND METHODS

#### *Animal collection*

Squid were obtained as a by-catch from a local mackerel fisherman and were caught in a floating box trap located on the south side of Herring Cove, near the mouth of Halifax Harbor in water of 16 m depth. The 35 mm mesh net box is "L" shaped with outer dimensions of 40 m and 24 m and is 12 m wide and deep at all points. The short leg of the "L" faces the shore and a 65 m "leader" of 115

mm mesh netting runs from the shore to a 3 m door, located at the inner angle, which can be closed after the catch enters. Squid were most frequently caught during spring tides. The wild population samples indicated in Figure 2A represent the majority, but not all of the seasons catches. Individual catches ranged from a few animals to several thousand, but small catches were most common in late May and early June when the squid are small enough to pass relatively easily through the "leader". The trap is certainly size-selective and this may account, in part, for the fact that over 90% of the squid were female since *I. illecebrosus* females are typically larger than males (Squire, 1967); however, the sex ratio changed little later in the season when males were much larger than early season females. The apparent steady growth during the season suggests that the squid belonged to a single modal class, as described by Squires (1967).

A total of 85 live *I. illecebrosus* was collected in three lots from the trap. For live collection, the floor and walls of the trap were raised to confine the squid to a small volume of water, and individual animals were transferred from the trap in buckets of water to  $60 \times 90$  cm fiberglass tanks filled to a depth of 30 cm. A maximum of 20 squid were held in each tank aboard the tending vessel with a continuous, copious flow of water for periods of about one hour during the 25 km trip to a dock near the Aquatron. The tanks were then transferred to a truck and supplied with air from a battery driven compressor during the 10 to 15 minutes needed to move them to the Aquatron pool. The portable tanks were lowered into the isolation pool (Fig. 1), floated into the main pool, and submerged to release the squid with minimum handling.

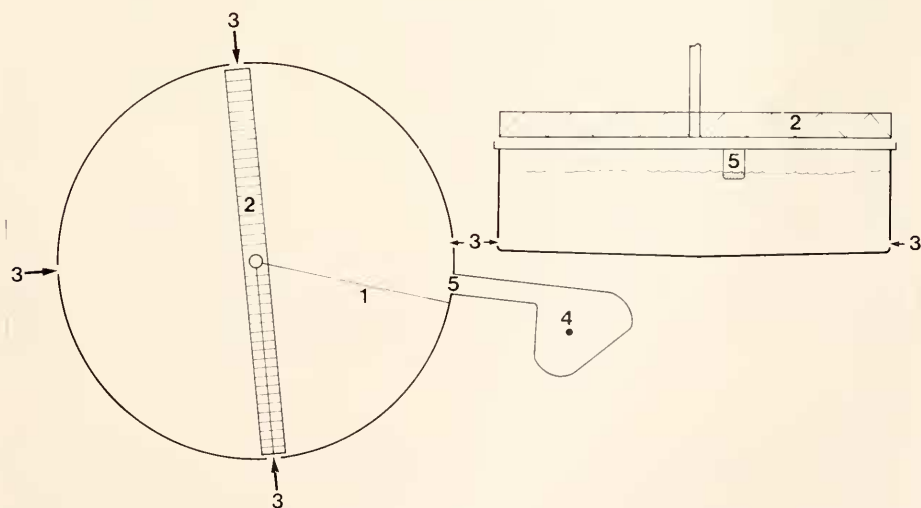


FIGURE 1. Plan and sectional views of the pool tank in the Aquatron Laboratory at Dalhousie University where squid were kept. Features discussed in the text are: 1) restricting curtain, 2) axial bridge, 3) water supply inlets, 4) drain in isolation pool, and 5) weir in connecting channel. Section is in the plane of the bridge.

### *Holding facility*

The indoor circular pool tank in the Aquatron Laboratory is 15.24 m in diameter, and its sloping bottom ranges in depth from 3.55 to 3.91 m at the center (Fig. 1). It was constructed of reinforced concrete, lined with multiple, sprayed coats of light olive-colored polyvinylchloride (PVC). Twenty-two underwater windows, each approximately 1 m square, are located around the perimeter of the tank providing viewing at all depths. The entire tank rests on neoprene blocks providing partial acoustical uncoupling. The connected isolation pool is 1.12 m  $\times$  1.8 m diameter. For the present study, the water level was held 0.5 m below maximum to prevent the squid from jetting into the scuppers, giving a total water volume of 580 m<sup>3</sup>. The 28 supply inlets, which were not in use, were blocked with 1 cm mesh PVC screening to prevent the squid from becoming trapped in the blind-ended pipes.

An axially suspended bridge spans the full width of the pool, and can be rotated mechanically from a remote control unit on the bridge to give access to any point on the surface. The pool is also equipped with a "vacuum cleaner" driven by hydrostatic pressure used to clean the bottom during long-term maintenance. For a more detailed description of the Aquatron Laboratory see O'Dor, Durward, Steadman and Balch, 1977.

### *Water supply*

Sea water was pumped from the Northwest Arm of Halifax Harbor through intakes located at a depth of 15 m, 0.7 m off the bottom. Although water quality is relatively high since the Arm is well flushed and since effluent and storm drainage is normally restricted to the surface layer, the water was routinely passed through four Graver pressure sand filters which were backwashed daily. Water flow into the main pool was through the bottom inlets opposite the isolation pool at rates of 100 to 150 liters/minute (Fig. 1). Drainage of the main pool via the isolation pool was over a weir providing surface skimming to remove feces and the warmest surface water. Temperature varied throughout the pool by less than 1° C and was generally 2 to 3° C above the intake temperature (Fig. 2). Temperatures varied from 17° C in September to 7° C in December, approximately the optimal temperature range for *I. illecebrosus* reported by Squires (1957). Although water is warmed 1 to 2° C as it enters the Laboratory there was no evidence of supersaturation in the pool; and although no measurements were made, O<sub>2</sub> levels were presumably similar to those in the ocean, since the water was not recycled and the volume was very large for so few animals. Salinity during these experiments varied from 29.5 to 32.0‰, but were very close to 30.0‰ during most of the period.

### *Lighting*

The pool is isolated from natural light except for three small windows above the water surface on one side which receive indirect skylight. This is a very small percentage of the artificial light provided by six 400 watt Sylvania Metalarc Lamps (400 BU-HOR) distributed uniformly over the surface at a height of 4 m. Light intensities in the lower 2 m of water where the squid usually stayed averaged 2 w m<sup>-2</sup> and varied from 0.04 to 5.5 w m<sup>-2</sup> in different areas. All of these values are

within the normal range near bottom in the area of capture (Platt and Irwin, 1968). A constant, daily photoperiod of 15.5 hours, equivalent to the longest days during the experiments, was used throughout the experimental period to minimize the influence of natural light. The metal-halide lamps have a long warm-up period which eliminates sudden increases in light which frighten squid (LaRoe, 1971), and the on-off cycles of the individual lamps were staggered at 15 minute intervals to simulate dawn and dusk. Low levels of indirect light, approximating starlight, were provided through the small windows during the dark period.

### *Feeding*

When squid were first placed in the pool, 100 to 200 live specimens of *Fundulus* spp., 5 to 10 cm in length, were also added. Most squid would feed on these fish within two hours of introduction. When the squid were accustomed to feeding in the pool, live specimens of *Fundulus* were tossed into a particular location one at a time until the squid came to feed. Once "trained" to this feeding area, they would take frozen smelt and chunks of frozen mackerel as they sank, although previously such food had been ignored. Food which fell to the bottom was never eaten, but could be easily cleaned from the small feeding area. Each squid received at least one 30 to 50 g chunk of mackerel per day, and certain individuals took two or three chunks before the others fed. Occasionally this diet was supplemented with live *Fundulus* which were preferred.

### *Handling*

One major difficulty in using the large pool for chronic experiments was recapturing the squid. Initially, squid could easily be lured to the feeding area with a live *Fundulus* on a string and taken from the water with a 35 × 40 cm bait net, 15 cm deep, made from 5 mm knotless nylon netting to minimize skin damage; but after one or two experiences, a squid would learn to distinguish free-swimming *Fundulus* from those with strings attached and would no longer approach. As a more general but tedious solution to the capture problem, a curtain was placed across the pool such that half of the curtain hung fixed to a rope running from the side, near the entrance to the isolation pool, to a swivel at the center of the bridge, while the other half hung from the rotating bridge itself (Fig. 1). Thus the curtain could be folded back on itself so that it blocked only half the width of the pool, giving the squid access to the entire pool. Rotating the bridge slowly through 350° forced all of the squid into a small wedge, opening at its outer edge to the isolation pool. The squid could be captured in the restricted area of the wedge or herded with hand nets into the isolation pool. The curtain was made of 0.05 mm thick black polyethylene sheeting with 2 cm holes every 50 cm in a grid pattern to allow water to flow through. The holes were punched with a cork borer so that flaps remained attached at the bottom; and these flaps, floating up to close the holes, gave the stationary curtain the appearance of a continuous wall. It was fitted to the sloping pool bottom and weighted. A fine netting curtain would have moved more rapidly through the water, but the risk of entanglement and skin damage would have been greater.

### Marking

A second difficulty with chronic experiments in a tank with large numbers of squid is identification, which was necessary when squid from two groups were in the pool at the same time and when individuals were measured and inspected more than once. Tags placed in any part of the body were either removed or resulted in self-mutilation, and fin notches tended to interfere with swimming and to become infected. The most effective marks were patterns of large dots tattooed onto the fins using India ink gravity fed through a 30 gauge needle on an open syringe. A few passes through the fin at a single location left a dot, which remained throughout the experiments, and was visible from above during capture or when viewing through underwater ports. Even when these dots occasionally became infected, they were still identifiable.

Tattooing was carried out under an anesthetic. After netting, the squid were transferred with wet hands to a  $40 \times 14 \times 14$  cm polyethylene container with a tightly fitting lid (a "bread saver") containing 3 to 4 liters of 3% urethane in sea water at approximately the same temperature as the pool. When respiratory movements stopped, after two to five minutes depending on temperature and animal size, the animals could be injected, tattooed or inspected by inserting a 2 cm diameter glass tube into the mantle opening. Animals were wrapped in wet towels during the procedures to prevent abrasions, drying and contact with dry surfaces. More complex surgical procedures of the sort previously carried out in *Octopus vulgaris* (O'Dor and Wells, 1973), including brain lesions and optic gland removal, could be carried out when the squid were left in the anesthetic until the "eye blink" reflex almost disappeared (usually three to four minutes after respiratory movements ceased). Recovery from light anesthesia occurred after the mantle cavity had been flushed with a gentle stream of sea water for four to five minutes, but recovery from the deeper anesthesia often took up to fifteen minutes.

## RESULTS

### Survival

Trapped squid were placed in the pool on three occasions in 1976: 15 squid on July 15 (group A), 35 squid on September 13 (group B), and 35 squid on October 24 (group C). Maximum survival times in each group, as indicated in Figure 2A, ranged from 32 days for group C to 82 days for group B. Although few group C animals were used for experiments, this group had the lowest mean survival time, 13 days. Realistic mean survival times cannot be given for groups A and B since the majority of the animals in these groups, including the longest lived, were killed for other experiments; however, minimum values calculated from deaths from all causes are 26 days and 35 days, respectively. Figure 3B illustrates how conservative these values are, since in group B only two of the deaths after day 50 were natural, and even these were remarkable. The second to last natural death was the only observed instance of cannibalism; the sole male in the group was found partially eaten. The last natural death was the first female to become fully mature. It had behaved unusually for several days prior to death, and 23% of its 442 g body weight was eggs in the oviducts ready to be laid.



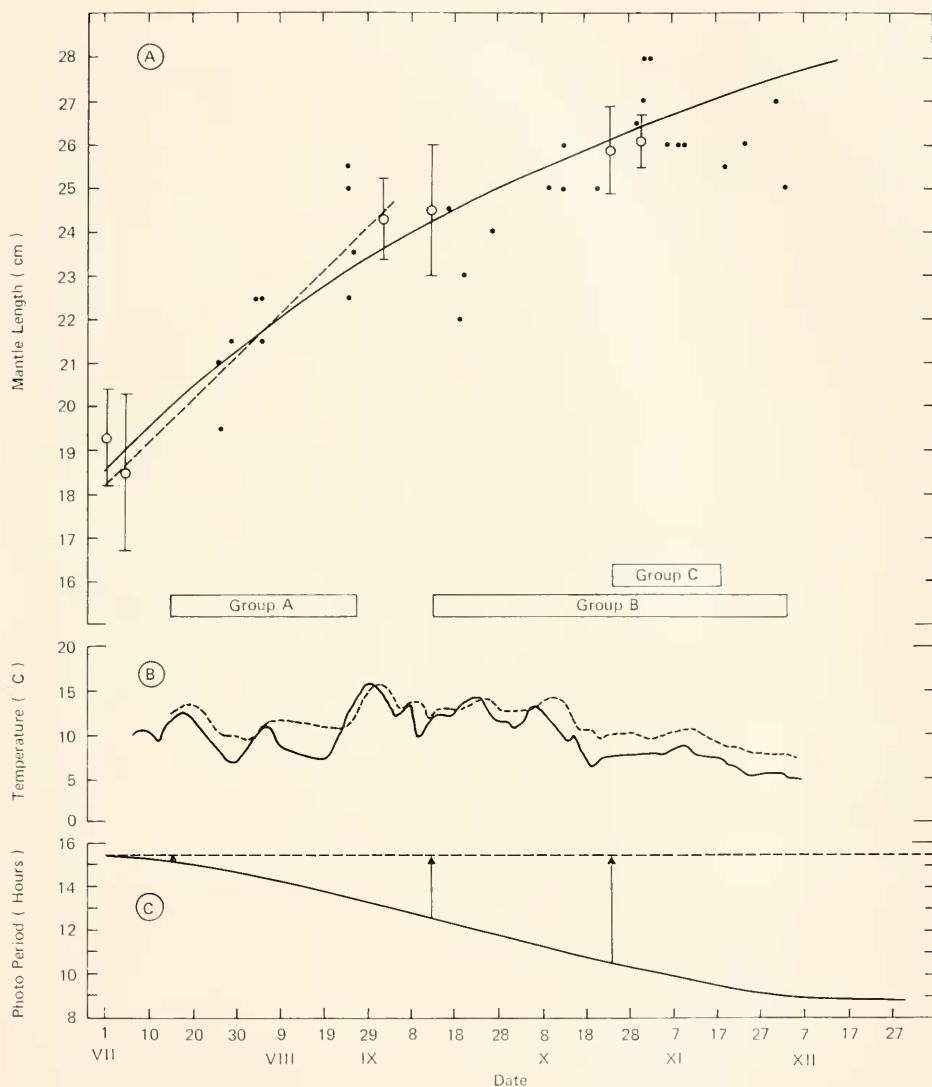


FIGURE 2. Physical conditions and growth of squid during the 1976 experiments. (A) Open circles and bars indicate mean mantle length (ML) and standard deviations for females taken from the wild. Solid circles indicate ML at death for animals from groups A and B which were kept in the pool during the indicated periods. The solid line is a growth curve for squid off Newfoundland redrawn from Squires (1967). The broken line is the regression of ML *versus* time for animals in group A. (B) The solid line is the water temperature at the intake and the broken line, that in the pool. (C) The solid line shows the natural photoperiod at 45°N latitude during the experiment (sunup to sundown), while the broken line indicates the photoperiod in the pool. The arrows show the change in photoperiod experienced by squid transferred from natural to experimental conditions.

*Growth and maturation*

The mean mantle lengths (ML) for samples of squid collected from the Herring Cove trap throughout the season, shown in Figure 2A, fit reasonably well on the growth curve given by Squires (1967) for *I. illecebrosus* off Newfoundland, suggesting that the population sampled in this study was fairly uniform, with a similar growth rate. Included in the figure are the mantle lengths at death of squid from groups A and B; these animals also appear to have grown at a similar rate. The linear regression of ML vs. time in the pool (broken line, Figure 2A) suggests a growth rate of 0.94 mm/day (correlation coefficient, 0.83) in group A, which is slightly greater than that reported by Squires (1967). However, the 15 squid in the pool were the total catch for that day so that no directly comparable data for ML at the start are available. A similar regression line can be drawn for group B for the first 50 days, but this line does not pass through the mean ML of the control group collected with B. In both groups early deaths of smaller squid appear to influence the regression so that calculated growth rates are slightly exaggerated; however, some growth is indicated in both groups. The average ML in group B was 1 cm larger at death than the average ML of controls killed on September 13. This was not a significant increase ( $P < 0.1$  by Student's *t*-test); however, for the squid which were in the pool for more than 40 days, the mean ML is 2 cm greater which is highly significant ( $P < 0.001$ , Mann-Whitney U-test). This is partly attributable to deaths of small animals, as indicated by a decreased variance in the later group, but the probability of the largest animals in this group having been present in the population at the start of the experiment is only 0.01.

The fact that the group B animals began to mature sexually after 30 to 40 days probably reduced their overall somatic growth. Nidamental gland length (NGL) was used as an index of sexual maturity, and as Figure 3A shows, it increased nearly four-fold in the pool in 50 days, while in animals from the sea the increase was only about 25% over the entire season. Eight of the fourteen squid remaining on October 24 were anesthetized and marked, and their mean NGL as measured through the mantle (to the nearest 5 mm) was  $70 \pm 11$  mm. The mean NGL at death over the next 40 days for this group was  $104 \pm 13$ . The average ovary weight ( $\pm$  s.d.) at death in this group was  $72 \pm 31$  g and that for squid from the sea at the same time was  $2.2 \pm 0.7$  g. Development of these large glands and ovaries was so extensive that they could easily be seen in the living squid from the observation windows. Somatic growth must have stopped as reproductive growth increased, since there was no further increase in ML among the marked squid; however, as they continued to feed, there were probably further increases in weight due to ovarian development. All of the animals eventually ceased to feed, even on live *Fundulus*, as the ovaries developed. We estimate from visual observations that ovaries weighed approximately 50 g when feeding stopped. Although no measurements were taken, the digestive system appeared to be greatly reduced in size when the fully developed animals were dissected.

Forty days in the pool produced a significant increase in NGL in group B, but squid from group A which survived for a similar period showed no increase. The four longest survivors in group C were killed for experiments after 20 to 30 days in the pool, when examination under anesthetic indicated that their ova were in

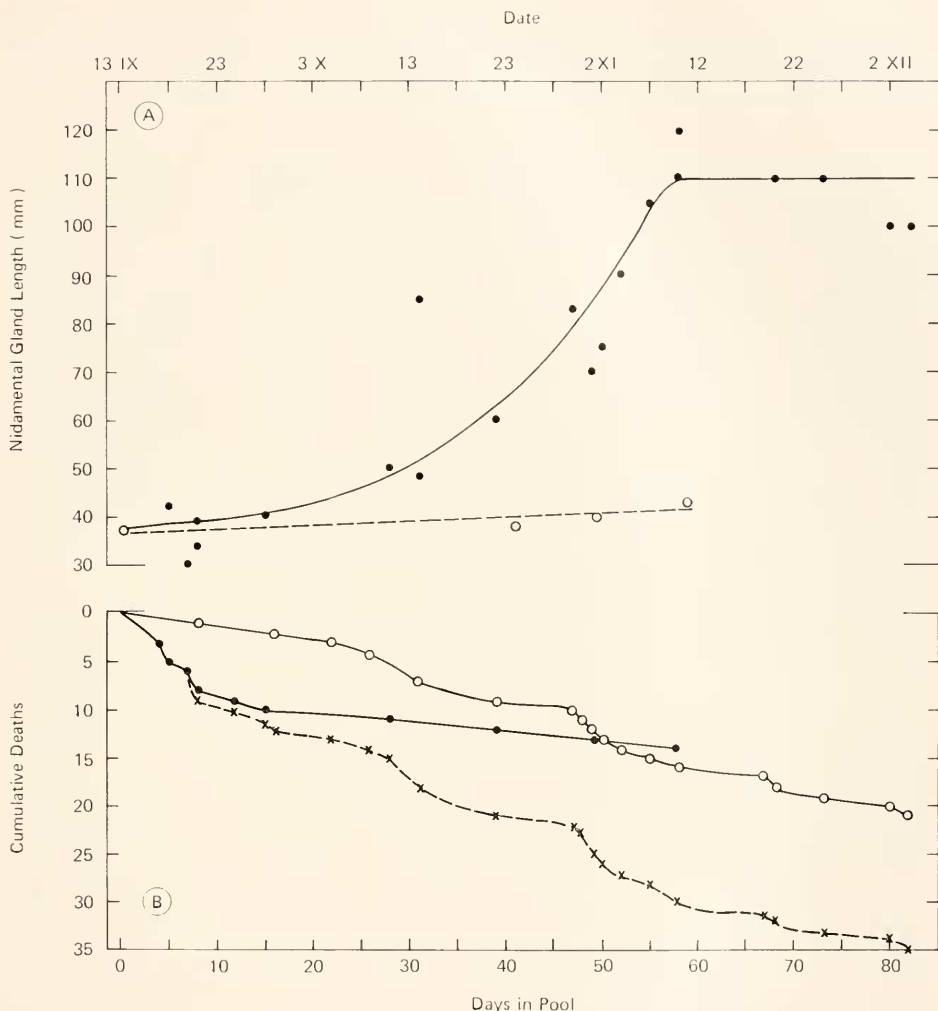


FIGURE 3. Deaths and nidamental gland lengths (NGL) at death for squid in group B. (A) Open circles are mean NGL for animals from the wild. Solid circles for NGL at death for group B animals in the pool. (B) The broken line is a record of total deaths in group B and is the sum of the natural deaths (solid circles) and animals killed for experiments (open circles).

the early stages of vitellogenesis. Average NGL in these animals was 58 mm, significantly ( $P < 0.01$  by  $t$ -test) larger than in comparable animals from the sea.

#### *Survival after surgery*

There were no deaths attributable to light anesthesia with urethane, tattooing or inspection; however, recovery from deep anesthesia was much less sure, particularly if the surgery required more than about five minutes. The recovery rate was about



50% in this latter group; animals which had had optic glands removed were killed after two days for other experiments, but the only animal to recover after removal of the vertical lobe of the brain survived for seven days.

#### DISCUSSION

The observations reported here agree with the suggestion (Summers *et al.*, 1974; LaRoe, 1971) that the major cause of early mortality in captive squid is skin damage. Squid have very delicate skin and apparently little capacity for healing when lesions are large. Squid which came into the pool with lesioned areas amounting to more than 2 to 3 cm<sup>2</sup> over their body surface never survived for more than a few days. However, individuals of *I. illecebrosus* and probably other species of squid can be maintained if they are brought to the laboratory without skin damage and are kept in conditions which minimize subsequent damage. It seems likely that the majority of the animals in groups A and B would have lived out their normal life spans had not the experimenters or prococious maturation interfered. When group C, which had the shortest mean survival time, was taken, the trap contained 5000 to 6000 squid, a much higher density than the other groups. Within 24 hours nearly all of the animals showed many skin lesions. The four that survived for more than ten days were essentially unmarked, fed well and were in excellent condition when killed. In previous years when specimens of *I. illecebrosus* and *Loligo pealei* (from predominately male schools of *L. pealei* present near Halifax in May and June before the major influx of *I. illecebrosus*) were collected for the pool by trawling, their condition and mean survival times were usually about the same as group C's; thus, results were similar to those reported by Summers *et al.* (1974). Survival times of trapped squid kept in the Aquatron tower tank (10.5 × 3.7 m diameter) were also low, probably as a result of large skin lesions which appeared after frequent bumping and circling of the tank with the point of the mantle and fin edges rubbing the walls. When frightened, squid jet backward, and in small tanks they hit the walls, which seems to frighten them further and leads to continued jetting. In small circular tanks they circle, abrading a small area on one fin, while in rectangular tanks they move along one side until they reach a corner where they bump for long periods, often abrading large areas on their posterior surfaces (Summers *et al.*, 1974). Of the two behaviors, circling probably damages a smaller skin area and seems the easier pattern to break. On this basis circular tanks seem slightly preferable.

Summers and McMahon (1974) have presented an elegant analysis of appropriate tank dimensions for *L. pealei* of various sizes, based on the concept of "squid mean free path". In the large pool, even when frightened, the squid rarely contacted the walls and generally remained in a fairly compact school slowly circling the pool or drifting back and forth one or two meters in from the wall. When an observer first appeared at the edge or on the bridge, the squid usually moved to the far side of the pool but would return if the observer made no further movements. After a period without food the school would actually approach a visitor, apparently in hopes of being fed. The response to an observer at a view port was similar, but on one occasion, when a large group of school children were observing through all of the view ports simultaneously, the squid withdrew to the center of the pool and became quite agitated. When the vacuum cleaner or nets

were used to remove debris from the pool bottom the squid would move away to maintain a reasonable distance, but were not greatly disturbed by the operation. The general impression was that the squid preferred to keep a certain minimum distance between themselves and any potential danger. This distance may also be a factor in determining the minimum size of tanks in which squid can "relax". From our results we can make the empirical observation that for *I. illecebrosus* of about 20 cm ML, a 3.7 m diameter is too small and a 15 m diameter is large enough.

If a 15 m tank is essential it should, at least, be possible to keep large numbers of squid in it. Summers and McMahon (1974) found no evidence of detrimental effects from crowding at densities of up to 16 squid/m<sup>3</sup>. This density would be equivalent to 9000 squid in the pool. The maximum number we have held at one time was 50 when group C was added to the remainder of group B. This addition had no ill effects on group B, and there was no evidence that any of the problems of group C were a result of interactions with group B. The squid from both groups immediately formed a compact school and those from group C fed normally on *Fundulus* until they became moribund. While feeding and cleaning up after 9000 squid is beyond contemplation, the fact that they remain in compact schools suggests that several hundred would not be an unreasonable number in a large pool. A good strategy for keeping many squid for long periods would be to construct a single circular tank as close to 15 m in diameter as was economically feasible and to fill it with trapped squid as they became available. Trapping is not as reliable a means of catching squid as trawling (Summers and McMahon, 1970), but the longer survival of trapped squid should more than compensate for the difference.

Aside from skin damage, the major factor limiting survival in squid appears to be sexual maturation, which in most studied cephalopods is associated with degeneration of organs and tissues not related to reproduction, cessation of feeding and ultimately with death shortly after spawning (Van Heukelem, 1973). This was not expected to be a problem, since about 95% of the animals collected were female and in several years of collecting locally throughout the season a maturing female *I. illecebrosus* had never been seen. This is not just a local phenomenon as there are few records in the literature of mature females, and it is thought that they move out into deeper waters before maturing (Squires, 1957; Mercer, 1973b). In fact, the use of surgical techniques previously applied to *Octopus vulgaris* to induce maturation was planned (O'Dor and Wells, 1973). This proved unnecessary since some factor in the pool environment caused rapid maturation of all animals in groups B and C surviving for more than about three weeks, although no maturation occurred in group A or in the wild population.

The only factor which has been specifically suggested to induce maturation in *I. illecebrosus* is starvation; Rowe and Mangold (1975) have shown that ovary and nidamental gland weights of unfed squid kept in the laboratory are significantly greater than those in wild populations within a few days, particularly in large animals (ML > 20 cm). They were, however, unable to feed their animals a diet adequate to prevent this reproductive development in the laboratory, so the question of what constitutes starvation is open. LaRoe (1971) suggests that loliginids should get 30 to 60% of their body weight in food daily and that they starve if the level is lower than 10-15%. Our squid received as much food as they would take in one daily feeding, a minimum of 17% of body weight in group A which grew at

an apparently normal rate without maturing. The minimum diet received by groups B and C was only 12.5% of body weight, since they were larger, but several squid (apparently the dominant members of the school; Arnold, 1962) consistently took up to three times this minimum and clearly grew. As all group B animals matured, starvation seems unlikely to be the common stimulus. While partial starvation cannot be ruled out as a contributing factor, some other feature of the laboratory environment, unrelated to food, must also have been involved in inducing maturation in our squid and possibly also in those of Rowe and Mangold (1975).

A second factor which might have influenced the maturation rate is temperature. The pool temperature was always a few degrees higher than that at 15 m in the Arm (Fig. 2), but how it compared with that experienced by the wild population is not known, since the vertical distribution of *I. illecebrosus* is not known with certainty. Most catch statistics for *I. illecebrosus* are based on incidental catches in ground fish trawls and prove only that the squid are present near the bottom in significant numbers during the day (Squires, 1957). Although there are no mid-water trawl data to confirm it, photographic records of vertical distribution of the squid kept in the 10 m tower tank indicate that *I. illecebrosus*, like many other squid (Clarke and Lu, 1975), make a diurnal migration to the surface at night. The average depth during the night, 1.6 m, was significantly ( $P < 0.01$ ) less than that during the day, 4.8 m (O'Dor *et al.*, 1977). Such diurnal migrations would expose the wild population in the area to temperatures varying from 2 to 5° C near the bottom to surface temperatures of up to 18° C (Platt and Irwin, 1968), but the average temperatures experienced by squid in the wild are unlikely to have been higher than those in the pool. Richard (1966, 1970) has shown that in *Sepia* higher temperatures give increased somatic and gonadal growth, a result different from that in the pool where there was a dramatic acceleration of gonadal development while somatic growth continued normally or declined. The temperature effect in *Sepia* seems to result from a generalized increase in metabolism rather than a selective activation of the gonads *via* the optic glands which normally control sexual maturation in cephalopods (Wells and Wells, 1959). The optic glands in our mature specimens were greatly enlarged and orange (characteristics of secretory activity), and the rate of gonadal development was comparable with that seen in *Octopus vulgaris* after the inhibitory nerves to the optic glands were sectioned (Wells and Wells, 1959). The pool environment seems to have "triggered" a rapid maturation phase rather than merely to have accelerated an on-going process. In any case, the pool temperatures were dropping just at the time of greatest development in groups B and C and were relatively high for group A which showed no maturation. The wild population which also experienced dropping temperatures at this time, did not mature, so that temperature cues cannot explain our observations.

The factor which has been most consistently linked to activation of the optic glands and maturation in cephalopods is light, usually as it relates to photoperiod (Laubier-Bonichon and Mangold, 1975; Richard, 1967; Wells and Wells, 1959). When Richard exposed six month old female *Sepia* to light regimes ranging from 1 hour light in 24 hours to 23 hours light in 24 hours there was no response for six months, but at the end of this refractory period all animals receiving 12 or

fewer hours of light matured within three months, while those receiving more than 12 hours of light matured at slower rates, apparently dependent on the photoperiod. We attempted to eliminate this variable by maintaining constant light intensity and photoperiod throughout the experiments; but in retrospect this may have been the wrong strategy, for while laboratory conditions remained constant those in nature did not. Group A was captured when the natural photoperiod was 15.1 hours and approximately this photoperiod was maintained in the pool. Group B's photoperiod changed from 12.5 hours to 15.5 hours on entering the laboratory and group C's from 10.5 hours to 15.5 hours. Thus, the two groups which matured received relatively large increases in photoperiod under experimental conditions. In comparing our results to those of Richard (1967), two interpretations are possible: first, Group A animals were refractory, but groups B and C were responding to the long photoperiod; or secondly, Groups B and C responded to the *change* in photoperiod, to which group A was not exposed. Both types of photoperiodic control are known to occur in other animals (Bünning, 1973).

It should be possible to determine which type of cue is the more likely natural one by looking at the life cycle of *I. illecebrosus*. Although the life cycle is not known completely, that part which is known appears to be quite regular. Squid of about 14 cm ML arrive on the Grand Banks and the Nova Scotian Shelf in late May, grow to about 28 cm ML and depart in early November (Squires, 1967 and personal observations). Females show virtually no sexual maturation during this period, but males may become fully developed. Squires (1967) has proposed a one year life cycle, based on rapid but not unreasonable growth rates, which requires that the young squid hatch in February. This cycle would be possible only if females could mate, spawn, and hatch their eggs in about 90 days between November and February. Females reached full maturity after as few as 55 days in the pool, so that if embryonic development requires less than 35 days, the cycle is feasible. There are no data on embryonic development of *I. illecebrosus*, but eggs of the closely related *I. coindetii* are the same size ( $1.0 \times 0.8$  mm) and develop in about six days at 20° C (Boletzky, Rowe and Aroles, 1973). Development times for cephalopod eggs are highly dependent on temperature as well as size, and Boletzky (1974) has shown for several species that with each 5° C decrease in temperature the time to hatching almost doubles. Although the eggs of *I. coindetii* from the Mediterranean did not develop at temperatures below 15° C (Boletzky *et al.*, 1973), extrapolation to 5° C, a temperature likely to be encountered in winter in bottom waters off the Grand Banks and Nova Scotian Shelf where *I. illecebrosus* presumably spawns, gives a time to hatching of 40 days. Development time for the eggs of *I. illecebrosus*, a species adapted to cold water (Roper, Lu and Mangold, 1969), is unlikely to be longer than this. If *I. illecebrosus* migrates to warmer waters to spawn, the time spent in migrating should be approximately balanced by decreased development time, supporting the proposed one year life cycle.

A simple assumption which would relate this natural cycle to photoperiodic stimulation of maturation in the pool is that migration and maturation are normally triggered in early November by the same cue. There is no actual increase in natural photoperiod until late December, but, as indicated in Figure 2C, the rate at which photoperiod decreases is reduced in early November at about the time migration begins. Rate of change of photoperiod is a cue known to affect sexual



maturation in birds (Morris and Fox, 1958). If squid have a similar system, it would explain the natural cycle, and an absolute increase in photoperiod, as occurred in the pool, would likely trigger any system sensitive to more subtle rate changes. This is the only obvious cue which can explain all of the observations. If the smaller animals of group A were refractory, light intensity would remain a possibility; but for animals which move freely vertically and are exposed to a wide range of light intensities, it seems an unlikely cue. In any case, the tank offered a choice of intensities in the natural range.

On this basis, the best strategy for preventing maturation in *I. illecebrosus* and reducing the resultant mortality would be the use of a constant photoperiod equal to that on the shortest day of the year, since the naturally occurring decreases in photoperiod do not induce maturation in the wild. Maintenance in continuous darkness or low levels of light is a second alternative, but the effects of such unnatural regimes on maturation is difficult to predict (Laubier-Bonichon and Mangold, 1975). If precocious maturation can be eliminated, long-term maintenance and chronic experiments with *I. illecebrosus* and possibly other squid are feasible, given a healthy starting stock and a large pool.

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#### SUMMARY

1. Female specimens of *Illex illecebrosus* of 20 to 28 cm mantle lengths lived, fed and grew in a 15 m diameter pool for periods up to 82 days; deaths during the first week were associated with skin damage during capture and those after the eighth week with precocious sexual maturation.

2. Under the conditions described, squid survived repeated capture and urethane anesthesia, as well as tattooing and surgical procedures. Techniques for handling are described.

3. Although the cause of early maturation is not certain, squid entering the pool were exposed to increased photoperiods, and a relationship is hypothesized between this stimulus and the natural November spawning migration.

4. The rapid sexual maturation observed and the small size of eggs ready to be spawned indicates that the one year life cycle previously proposed for *Illex illecebrosus* is feasible.

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